## REMARKS/ARGUMENTS

This Amendment accompanies a Request for Continued Examination (RCE) and addresses issues presented in the Final Rejection of October 23, 2007.

Discussion of Amendment of the Claims

The previous claims have been revised to reinstate polynucleotides and polysaccharides as options for the macromolecular principle but also to specify that the pharmaceutical composition is one intended for oral administration and includes a solubilization aid. In order to provide a coherent set of claims, the previous claim set has been withdrawn and a new claim set added. The relationship between the previous claims and the new claims presented herein is as follows:

| Previous Claim | New Claim | Previous Claim | New Claim |
|---|---|---|---|
| 30 + (b) of 31 | 59 | 46 | 73 |
| 31 (c) | 60 | 47 | 74 |
| 32 | 61 | 48 | 75 |
| 34 | 62 | 49 | 76 |
| 35 | 63 | 50 | 77 |
| 36 | 64 | 51 | 78 |
| 37 | 65 | 52 | 79 |
| 38 | 66 | 53 | 80 |
| 39 | 67 | 54 | 81 |
| 40 | 68 | 55 | 82 |
| 41 | 69 | 57 | 83 |
| 42 | 70 | 58 | 84 |
| 43 | 71 | 59 | 85 |
| 44 | 72 | 86-89 | New |
| 46 | 73 | | |

The claims have been revised to reinstate references to polynucleotides and polysaccharides, and attached is Annex A, giving details of further supporting experimental data

prepared by the applicant. The examiner is referred to the passages at page 6, lines 12-13, page 7, line 7 and page 8, line 32 of the description.

Polynucleotides and polysaccharides have been reinstated in the claims as options for the macromolecular principle, based on e.g. original claim 9. Consistent with this previous dependent claims 38 and 52 have been reinstated as new claims 66 and 79.

Independent claims 30 and 31 (now new claims 59 and 60) have been reformatted as one independent and one dependent claim.

New dependent claims 85 to 89 have been added, based on page 7, lines 7 to 8 and page 8, line 32 of the description. These claims focus on preferred polynucleotides and polysaccharides.

GLPI and GCSF have been added to the list of possible macromolecular principles in previous claim 42 (new claim 70) for consistency with claim 53 (new claim 80).

Some minor corrections have been made to previous claims 40, 49 and 58 (new claims 68, 76 and 84).

Interview Summary of Interview of May 17, 2007

The current Official Action indicates that the Interview Summary of the meeting held on May 17, 2007 was not placed in the file by the former examiner. This omission, counsel believes, works to applicant's disadvantage in that the current examiner did not have the benefit of the events that transpired during the interview with the inventor, the undersigned, the previous examiner and the examiner's supervisor Cecilia Tsang who signed the Interview Summary.

For completion of the record, I attach a copy of the Interview Summary handed to the undersigned on May 17, 2007 at the conclusion of the interview. Counsel also states that at the conclusion of the interview on May 17, 2007, it was certainly the inventor's understanding as well as that of the undersigned that the submission of May 29, 2007 placed the claims of this application in condition for allowance and that no further information, claim amendments or data was required.

Turning now to the issues raised in the current Official Action, the Office Action essentially raises two objections -- one under written description and another under obviousness. In both cases the Examiner Audet (the current Examiner) repeats the previous objections raised

by Examiner Khanna in the Office Action issued on February 27 2007, although it is noted that the objection under written description has now been expanded to apply to all pending claims.

The Office Action (page 3)

The comments further below address the points originally raised by Examiner Khanna, which are repeated by Examiner Audet on pages 4 to 8 of the Office Action dated October 15 2007. Before that, though, the following comments are offered with respect to the additional points made by Examiner Audet at page 3 of the present Office Action.

Firstly, applicant draws attention to the following comments:

> *"Namely, that the prior art of record... ...would not have motivated the skilled artisan to arrive at an enteric coated composition comprising ANY peptide or ANY derivative/analogue of certain known peptide genuses... ...AND a known aromatic alcohol (which inherently works as a absorption enhancer in the product)."*

The Examiner has apparently not appreciated the fact that the aromatic alcohol for use in the compositions of the present invention must be present in an amount by weight greater than or equal to that of the active macromolecular principle. This is a requirement of independent claim 59.

This aspect of the invention is clearly explained at page 2, lines 1-12. Thus, it is acknowledged that the aromatic alcohols for use in compositions of the present invention are known for use in pharmaceutical practice as antioxidants. However, it was completely unknown and indeed surprising to find that these aromatic alcohols can enhance the absorption of macromolecules in the intestine. Following on from this finding, the present applicant has formulated the aromatic alcohols into a pharmaceutical composition with a macromolecular principle, in an amount by weight greater than or equal to that of the macromolecular principle. Claim 59 also requires the compositions to be formulated so as to be suitable for oral administration. On top of that, they must also have an enteric coating which becomes permeable at a pH of 3 to 7. This will mean that the contents are released in the small intestine, as explained at page 9, lines 20-24.

It is acknowledged that the aromatic alcohols have previously been used in pharmaceutical practice as antioxidants. However, in this context it is usual to employ them in very low concentrations. An example of this may be seen in, for instance, WO 02/22158 of

Chakravorty *et al.* This is one of the documents cited in the Office Action. It discusses pharmaceutical compositions that can contain an antioxidant. The preferred antioxidant is the aromatic alcohol alpha-tocopherol (*see* page 11, lines 11-12). The preferred quantity of this antioxidant in the compositions is 0.0008 – 0.0009 % (*see* page 9, lines 3-4). This is in contrast to the immunosupressant agent, which is present in amount of 2 – 10 %. This is in stark contrast to the compositions of the present invention, which require the aromatic alcohol to be present in an amount by weight greater than or equal to that of the active macromolecular principle.

Accordingly, the skilled person would have had no motivation to employ the aromatic alcohols defined in claim 59 in the high quantities required, in an oral pharmaceutical with an enteric coating that becomes permeable at a pH of from 3 to 7. Any suggestion to the contrary can only be made with the benefit of hindsight, which is of course not permitted when assessing obviousness. This point is further reinforced by the fact that the aromatic alcohols for use in the compositions of the present invention had been used in pharmaceutical practice for at least twenty years (*see* page 2, lines 3-5 of the present application). If it was in any way obvious to use such aromatic alcohols to formulate a composition of claim 59, then surely it would have long since been done by now.

A crucial point here is that without the knowledge of the functional effect of the aromatic alcohol, i.e. that it would enhance the absorption of the active macromolecular principle, the skilled person would simply have had no motivation whatsoever to formulate such a composition. It is therefore respectfully submitted that the Examiner's assertion that "*the functional effect of the alcohol in the product bears no patentable weight*", which seems to suggest that the effect of the alcohols can be dismissed when considering obviousness, is not sound.

Further down page 3 of the Action, the Examiner suggests that it would have been "a matter of routine optimization" to add the aromatic alcohols defined in previous claim 30 to a pharmaceutical preparation in order to enhance absorption. With respect, the applicant cannot agree with this.

Firstly, the skilled person would not have known that the aromatic alcohols used in the compositions of the present invention would have had that effect. Indeed, as noted above,

despite the fact that they have been used in pharmaceutical practice for at least twenty years, this effect has not been appreciated by anyone before the present applicant.

Secondly, it would not have been a routine matter to use the specific aromatic alcohols defined in claim 59 as absorption enhancers in compositions, in light of the known uses of alcohols for this purpose. It is not clear which known alcohols the Examiner has in mind at page 3 (sixth line from bottom) of the Action. By way of example, therefore, we consider the known aromatic alcohols phenyl ethanol and benzyl alcohol.

The use of benzyl alcohol and phenyl ethanol to increase the permeability of a barrier layer of intestinal cells has been discussed before in the prior art (*see* e.g. page 1, lines 16-20 of the present application). These compounds do not fall within the definition of the aromatic alcohol absorption enhancer used in the compositions of claim 59. *Inter alia,* this is due to the fact that the aromatic alcohols defined in claim 59 necessarily feature hydroxy substituent(s) attached directly to the aromatic ring. Being attached directly to an aromatic delocalized pi system instead of a fully saturated hydrocarbon group does, of course, have a profound effect on the functionality of the hydroxy group. As the hydroxy substitutent is the prominent functional group in benzyl alcohol and phenyl ethanol, the properties of the molecule as a whole will obviously be highly sensitive to any changes in its functionality.

Furthermore, it is essential that the aromatic alcohols used in claim 59 also carry an additional substituent, directly attached to the central aromatic ring, and that this substituent is *para* to a hydroxy group. On top of that, one or more additional substituents must also be present. This is in contrast to benzyl alcohol and phenyl ethanol, which feature just one substituent (to which the hydroxy group is attached) on the central aromatic ring. The introduction of one or more additional groups in this way will clearly have both sterically and electronically significant effects, and so will further alter the properties of the molecule as a whole.

Merely by way of example, two basic fundamental differences between benzyl alcohol (or phenyl ethanol) and the aromatic alcohols as defined in claim 59 are explained in the present application as filed:

> "...phenoxyethanol, phenyl ethanol and benzyl alcohol,... ...are all liquids at room temperature, and can be readily dissolved in aqueous media" (page 1, lines 11-15)

and

> "...propyl gallate, butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA)... ...are all solids which are sparingly soluble in water, thus making it difficult to incorporate them into water-based pharmaceutical formulations in high concentrations, and also preventing them from being available in soluble form to act as enhancers at elevated concentration when the formulation is dispersed in the lumen of the intestine, or close to any other mucosal surface where permeation enhancement is required." (page 2, lines 1-12)

Thus, the benzyl alcohol and phenyl ethanol have completely different properties and forms at room temperature to the aromatic alcohols used in the compositions of claim 59. These differences are discussed further below, in the comments that address the obviousness objection set out on pages 6-8 of the Office Action.

The nature of these differences and their fundamental effect on the properties of the molecule as a whole are such that it is simply not credible to suggest that any skilled person would have even contemplated (let alone thought routine) using the aromatic alcohols defined in claim 59 as enhancers of the absorption of macromolecules based on uses known for benzyl alcohol and phenyl ethanol.

In view of the significant differences between the aromatic alcohols used in claim 59 and those that have been used before, it is submitted that a skilled person would not have expected the aromatic alcohols defined in claim 59 to enhance the absorption of an active macromolecular principle in the way the present applicant has demonstrated. Accordingly, the results achieved by the present applicant were unexpected. Further, as the skilled person would have had no motivation to formulate a composition of claim 59, it is respectfully submitted that claim 59 involves an inventive step and the invention it claims is not obvious.

Written Description (pages 4 – 6)

The Action asserts that the written description requirement is not met for any of the then-pending claims. With respect, the applicant disagrees, for the reasons set out below.

One of the original reasons given as to why the Examiner thought the claims should be restricted is that he did not think there was "any core structure present in the 'active macromolecular principle', or the disclosure of a specific structure that will give rise to an

intended activity." He also argued that where the present application discusses the variety of macromolecules that could be used, a lack of clarity results. With respect, it is believed that the Examiner is not focusing on what the applicant has actually invented. The applicant has discovered how the absorption of macromolecules may be enhanced, and provides compositions and methods for achieving this (*see*, for instance, page 2, lines 33-36 and page 3, lines 13-17). The ability to enhance delivery in this way is generally applicable to all macromolecules and all methods of treatment that involve administering them.

It is an internationally recognized principle of patent law that an applicant need not exemplify every single possible specific embodiment falling within a given genus in order to be entitled to a claim covering the whole of that genus. This is especially true in respect of features of the invention that are not essential to the invention's working. Indeed, demanding even the listing and description (let alone exemplification) of all possible embodiments in this situation is entirely unrealistic and would quickly lead to impractical drafting considerations and result in completely unintelligible patent specifications. For instance, if one has invented a new wing mirror for a car, it should not be necessary to explain in the resulting patent application how the car's engine works. To expect otherwise would place an unreasonable burden on both the applicant drafting his patent specification and the general public hoping to read and understand it.

Rather, the addressee of the patent specification must be assumed to have a reasonable level of skill and knowledge of the art. It is then unnecessary to describe in detail the non-essential aspects of the invention and it is practical (and indeed preferable) to explain that variation within the whole of the genus is tolerable. Nonetheless, it can also assist matters if the applicant can demonstrate success in specific examples from which one can arrive at the claimed genus by reasonable extrapolation. That is what the applicant has done in the present case.

As explained at page 2, lines 33-36 of the present application, the present applicant has found that "surprisingly, aromatic alcohols such as propyl gallate, BHT, BHA and analogues and derivatives thereof are capable of enhancing the passage of macromolecules across mucosal barriers by increasing the physical permeability of the mucosal cells." (emphasis added). As this effect is physical and related to the macromolecule's size (*see* also page 2, lines 29 to 32), it is

independent of the chemical structure of the macromolecule and so the present invention is expected to work for any macromolecule.

Notwithstanding this, the applicant included in the application as filed details of the successful incorporation of a selection of very different macromolecules into a composition of the present invention. More specifically, the Examples section of the present application describes the successful preparation of compositions comprising insulin (Examples 2 and 3), calcitonin (Examples 4, 5 and 10-12), parathyroid hormone (Examples 6-8) and human growth hormone (Example 9). Further, different methods of formulating the macromolecules used are given (c.f. Examples 7 and 8, for instance), and different solutions are used for carrying the macromolecules (c.f. Examples 2 and 3, for instance).

The Examiner's attention is further directed to Annex A provided herewith. Annex A provides details of further experiments carried out by the present applicant. This is evidence of what was already clearly explained in the application as originally filed (*see* page 6, lines 4-13, page 7, line 7 and page 8, line 32), namely that the macromolecular principle for use in compositions of claim 59 can be a polynucleotide or a polysaccharide.

Consider, for instance, insulin and calcitonin. These two macromolecules have no homology whatsoever. They possess completely different sequences, tertiary structures, isoelectric points and molecular weights. As the Examiner will appreciate, this list of differences could be continued further, but it is believed unnecessary to include that information here. Suffice to say that there are significant differences between the properties of these two macromolecules. Accordingly, the exemplification of these two macromolecules (let alone the others) clearly overcomes any suggestion that the efficacy of the method is related to any particular chemical characteristic that limits the invention to just one particular subset of macromolecules. Nonetheless, the applicant also included details of the successful formulation of compositions comprising further (and again very different) macromolecules, to further reinforce the invention's general applicability to all macromolecules.

Moreover, as noted above, the applicant clearly explained in the application as filed that the macromolecular principle can also be, *inter alia*, a polynucleotide or polysaccharide. The experimental results described in Annex A provide evidence to prove that this explanation provided in the application as filed is sound.

Polynucleotides and polysaccharides have even greater fundamental differences compared to each other, and also to insulin, calcitonin, and the various other macromolecular principles exemplified in the application as filed. In particular, they demonstrate the same numerous fundamental differences in terms of biological and chemical properties as the polypeptides do to each other, but go one step further because their backbone structures are different too. Thus, one simply cannot conceive of any particular chemical or biological feature or function shared by all of these different macromolecular principles that should call for the invention to be limited to just one particular subset of macromolecular principles. Indeed, the only feature they share is a physical one, namely their size, i.e. the fact they are actually macromolecules.

The applicant could, of course, have carried out and described in the application as filed countless experiments, using a number of different macromolecules that could be simply vast. However, this would have been an unnecessary waste of both the applicant's time and resources, and the time the addressee of the patent specification (i.e. a person of skill in the art) would have to spend reading it. Any skilled person would appreciate that, given the significant differences between the above-mentioned macromolecular principles, the invention would work with any macromolecule.

Furthermore, the applicant could also have carried out countless experiments demonstrating how each of the vast number of macromolecules may be used to treat the particular diseases against which they are already known to be effective. However, this is simply not within the remit of the present application. The present invention provides compositions and methods for increasing the absorption of macromolecules. As explained above, this invention is generally applicable to all macromolecules, and self-evidently is therefore equally generally applicable to all methods of treatment involving the administration of those macromolecules. Thus, there is no need to indicate any particular activity required of the macromolecule or of efficacy against any particular disease. The efficacy of the particular agents will often already be a matter of public record.

As is clear from the above discussion, the genus of macromolecules does not require identification of a common complete or partial structure, chemical property, functional characteristic, structure/function correlation or production method. All that is required is for it to

actually be a macromolecule, i.e. to be of sufficiently large physical size. Accordingly, the suggestion that there should be a "core structure present in the 'active macromolecular principle', or the disclosure of a specific structure that will give rise to an intended activity" is, with respect, believed to be simply wrong.

Notwithstanding all of this, in the interests of expediting allowance, the present claims have been limited to refer only to certain specific macromolecular principles, namely polypeptides or proteins, polynucleotides and polysaccharides. It was explained quite clearly in the application as filed that these are all suitable macromolecules for use according to the present invention, and for each type details of experiments have been provided as evidence.

It is respectfully submitted that, in view of the comments above and faced with the evidence discussed, it is simply not credible to maintain that the present applicant did not have full possession of the claimed invention at the time of filing. It is therefore believed that the written description requirement is satisfied.

Non-Obviousness (pages 6 – 8)

It would not have been obvious to use the aromatic alcohols specified in claim 59

The Examiner acknowledges that the aromatic alcohols specified in previous claim 30 and in current claim 59, namely butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) and propyl gallate (PG), are not disclosed in New ('28436) (this document is used as the starting point in the Examiner's obviousness objection). Setting aside, for the moment, the specific points raised in the Office Action, there are basic fundamental technical reasons why it would not have been obvious to use the aromatic alcohols of present claim 59. That is because of the significant differences that exist between them and those discussed in New ('28436).

New ('28436) exemplifies the specific aromatic alcohols benzyl alcohol (Ph-$CH_2$-OH), phenoxy ethanol (Ph-O-$CH_2$-$CH_2$-OH) and phenyl ethanol (Ph-$CH_2$-$CH_2$-OH), *see* e.g. page 2, lines 23-25. These aromatic alcohols are hydrophilic, *see* e.g. claim 1 at page 20, line 7 and page 2, lines 11-12. They are also are water-soluble, *see* e.g. Example 15, which describes the testing of the flow of these compounds between two "aqueous compartments" (page 18, line 24) separated by a porous membrane.

The aromatic alcohols specified in present claim 59, on the other hand, are highly lipophilic and very water-insoluble. This is due to key differences in chemical structure. Thus,

unlike the aromatic alcohols mentioned in New ('28436), BHT, BHA and PG all have unsubstituted terminal alkyl groups. More specifically, BHT and BHA have tertiary butyl groups and PG has a propyl group. Evidently these organic hydrophobic moieties contribute towards BHT, BHA and PG being lipophilic and water-insoluble. Evidence for these properties may be found, for instance, in the Handbook of Pharmaceutical Excipients. Copies of the entries for BHT, BHA and PG from the second edition of this book have already been provided as part of the IDS filed on April 2, 2007. Nonetheless, further copies are provided here for ease of reference. As can be seen, BHT and BHA are described as "practically insoluble in water" while PG has a solubility in water (at 20 °C) of only 1 in 1000.

Further evidence of the fundamental differences between the compounds used in New ('28436) and those used in the present invention is in the physical state of the various compounds at room temperature. Thus, benzyl alcohol (for instance) is a liquid whereas BHT, BHA and PG, on the other hand, are all solids. This is evident from the melting points of BHT, BHA and PG, which are, respectively, 70, 47 and 150 °C (*see* again the extracts from the Handbook of Pharmaceutical Excipients).

Against this background it is simply not credible to suggest that a skilled person would consider using the aromatic alcohols defined in claim 59 in place of those used in New ('28436).

The Examiner's reasoning is not sound

Notwithstanding the points made above, the following comments are offered in respect of the points made by the Examiner.

First of all, it seems that the Examiner's argument is based on the idea that it would be obvious to start with a composition from New ('28436) and then modify it in view of the other prior art documents cited to arrive at present claim 59. However, such modification would have been contrary to the actual teaching of New ('28436) itself. New ('28436) states at page 2, lines 20-21 that "Preferably, there are no hydroxyl groups attached directly to the ring of the hydrophilic aromatic alcohol". However, the aromatic alcohols mentioned in present claim 59 (namely BHT, BHA and PG) all feature one or more hydroxy groups attached directly to the central benzene ring. Thus, the teaching of the very prior art document the Examiner uses as a starting point directly and explicitly discourages the very combination the Examiner suggests is obvious. Moreover, it even teaches directly away from the Examiner's combination.

Nonetheless, considering the Examiner's objection as a whole, he seems to suggest the following chain of reasoning. BHT is known as an antioxidant (Chakravorty and Ivanovic). It is known that the amount of ionized vs unionized antioxidant determines pH (Ivanovic). It is known to use a pH buffer to enhance absorption in the digestive tract (New '748). Therefore it is obvious to use the antioxidant BHT to determine the pH and thereby enhance absorption. With respect, the applicant cannot accept this chain of reasoning.

When discussing Chakravorty it seems the Examiner has deliberately referred (page 9, last paragraph) to (a) benzyl alcohol (on of the compounds used in New ('28436)) as well as (b) BHT and PG, in order to suggest some kind of equivalence between them, i.e. that they can both equally well be added to pharmaceutical compositions.

The fundamental differences between the compounds used in New ('28436) and those defined in claim 59 of the present application have already been discussed above. In addition to that, though, one need look no further than elsewhere in Chakravorty itself to discover that equivalent considerations do not apply to (a) and (b). This is clear from, for instance, claims 14 and 15. Thus, claim 14 indicates that the preferred amount of benzyl alcohol preservative is "about 0.5 to 1 % by weight". This is in stark contrast to claim 15, which indicates not only that the preferred antioxidant is alpha-tocopherol (i.e. not BHT or PG) but also indicates a preferred concentration of "about 0.0008 to 0.0009 %". Not only would such concentrations be too small for any demonstrable enhancement of absorption, clearly, they would lead the skilled person directly away from a composition of present claim 59, namely one containing the aromatic alcohol in an amount greater than or equal to that of the active macromolecular principle.

In fact, the reference to BHT, BHA and PG in Chakravorty is just one example of the normal prior art use of these compounds as antioxidants. This is explained by the applicant in the paragraph bridging pages 4 and 5 of the International application as published.

The Examiner's reasoning also suggests that the teaching of Ivanovic would enable a skilled person to control the pH of a solution using e.g. BHA in a way that is compatible with the compositions used in New ('28436) and with the aromatic alcohols selected from Chakravorty. With respect, the applicant disagrees. In fact, Ivanovic is not concerned with using compounds to control pH; rather, Ivanovic concerns the effect that pH has on, *inter alia*, antioxidants such as BHT. This is clear from e.g. the title: "Effect of pH on...". As explained in the "Conclusion"

section of Ivanovic (page 656), what the article purports to teach is a method of quantitatively and qualitatively analyzing preservative-antioxidants in pharmaceutical formulations by HPLC. Ivanovic does not teach the skilled person how to control pH by using antioxidants such as BHT as a buffer, even setting aside the various incompatibilities (discussed above and below) in the combination the Examiner suggests.

Notwithstanding this, even if the skilled person did attempt to use BHT to control the pH of a pharmaceutical composition in an effort to enhance absorption of proteinaceous materials in the way described by New ('748), he would quickly run into difficulty. That is because New ('748) suggests that the absorption of a pharmaceutical composition is enhanced by adjusting the pH of the gut to 7.5 to 9 (*see* e.g. column 2, lines 47-59). In order to do this, the pH of the formulation itself needs to be alkaline when dissolved in water. The Examiner appears to suggest that this is possible because Ivanovic indicates that in methanol/water mixtures the pKa of BHT ranges from 7.5 to 9. However, in no way can that lead on to the conclusion that BHT could be used to achieve a pH of 7.5 to 9 in the gut. That is because in Ivanovic the pH of the compositions discussed (including BHT) range from 2.98 to 3.78 (*see* e.g. Table III), i.e. the compositions are strongly acidic. Thus, it is simply not credible to suggest that BHT could be used to increase the pH of a solution to 7.5 to 9, let alone having any hope that it might indirectly enhance absorption by improving the therapeutic index of the bile salts present in the gut.

Moreover, it is clear from the application as filed that the present invention enhances absorption in an entirely different way. In New ('748) the absorption is enhanced by increasing the therapeutic index of the bile salts already present. However, in the compositions of the present invention the aromatic alcohol absorption enhancer exerts an effect on cells independently of bile salts. This is abundantly clear from the Examples, which show an enhanced flow of materials across a cell monolayer (*see* page 12, lines 30-32) in the absence of any bile salts. The Examples also describe compositions with a pH of e.g. 3.15 (Example 2) or 3.36 (Example 3), i.e. strongly acidic, in stark contrast to the alkaline conditions discussed in New ('748), further reinforcing the incompatibilities inherent in the Examiner's reasoning.

In view of the comments above it is respectfully submitted that the reasoning presented by the Examiner to suggest that the composition of claim 59 is obvious from the prior art is not sound. Accordingly, the rejection should be withdrawn.

Notwithstanding the above comments, claim 59 has been worded such that the composition covered is an oral pharmaceutical composition, i.e. a composition suitable for oral administration. It has also been amended to specify that the composition has an enteric coating which becomes permeable at a pH of from 3 to 7. This focuses claim 59 on compositions which necessarily make use of the specific unexpected advantages discovered by the present applicant.

For the above reasons it is respectfully submitted that the claims of this application define inventive subject matter which is enabled by the description of the invention when viewed by one of ordinary skill in the art. Reconsideration and allowance are solicited. Should the examiner require further information, please contact the undersigned.

Respectfully submitted,

**NIXON & VANDERHYE P.C.**

By: _____

Arthur R. Crawford
Reg. No. 25,327

ARC:eaw
901 North Glebe Road, 11th Floor
Arlington, VA 22203-1808
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

# ANNEX A

**Example II(A) Preparation of formulation containing Poly-uridylic acid single-stranded RNA, propyl gallate and sodium taurocholate**

Sodium taurocholate in an amount of 750mg is mixed with 350mg of propyl gallate in a glass vial and 1.05ml of distilled water are added, to give a clear colourless solution on warming at 37 degC. To 1mg of poly-uridylic acid single-stranded RNA 0.5ml of the taurocholate/PG solution is added and incubated at 37 degC. A clear solution is rapidly obtained, with a pH of 3.15. The contents of the vial are frozen rapidly with shaking and lyophilised overnight. The following day a dry solid is obtained. 500ul of phosphate buffered saline is added to the solid. A clear solution forms rapidly.

**Example II(B) Preparation of formulation containing Poly-uridylic acid single-stranded RNA, propyl gallate and sodium taurodeoxycholate**

A procedure identical to that described in example II(A) was performed, except that sodium taurodeoxycholate was employed instead of sodium taurocholate, with identical results.

**Example II(C) Preparation of formulation containing Polyuridylic acid-Polyadenylic acid double-stranded RNA, propyl gallate and sodium taurodeoxycholate**

A procedure identical to that described in example II(B) was performed, except that polyuridylic acid-polyadenylic acid double-stranded RNA was employed instead of poly-uridylic acid single-stranded RNA, with identical results.

**Example II(D) Preparation of formulation containing Poly-cytidylic acid single-stranded RNA, propyl gallate and sodium taurocholate**

A procedure identical to that described in example II(A) was performed, except that poly-cytidylic acid single-stranded RNA was employed instead of poly-uridylic acid single-stranded RNA, with identical results.

**Example II(E) Preparation of formulation containing Poly-cytidylic acid single-stranded RNA, propyl gallate and sodium taurodeoxycholate**

A procedure identical to that described in example II(B) was performed, except that poly-cytidylic acid single-stranded RNA was employed instead of poly-uridylic acid single-stranded RNA, with identical results.

**Example II(F) Preparation of formulation containing Poly-guanylic acid single-stranded RNA, propyl gallate and sodium taurocholate**

A procedure identical to that described in example II(A) was performed, except that poly-guanylic acid single-stranded RNA was employed instead of poly-uridylic acid single-stranded RNA, with obtention of a homogenous translucent solution after re-dissolution of the lyophilised powder.

**Example II(G) Preparation of formulation containing Poly-guanylic acid single-stranded RNA, propyl gallate and sodium taurodeoxycholate**

A procedure identical to that described in example II(B) was performed, except that poly-guanylic acid single-stranded RNA was employed instead of poly-uridylic acid single-stranded RNA, with identical results.

**Example II(H) Preparation of formulation containing Low-Molecular weight heparin, propyl gallate and sodium taurocholate**

A procedure identical to that described in example II(A) was performed, except that 20mg of low-molecular weight heparin was employed instead of 1mg poly-uridylic acid single-stranded RNA, with obtention of a homogenous clear solution after re-dissolution of the lyophilised powder.

# Handbook of PHARMACEUTICAL EXCIPIENTS

Second Edition

*Edited by*
**Ainley Wade** and **Paul J Weller**

American Pharmaceutical Association
Washington

The Pharmaceutical Press
London

1994

# Propyl Gallate

## 1. Nonproprietary Names

BP: Propyl gallate
USPNF: Propyl gallate

## 2. Synonyms

E310; gallic acid propyl ester; *Progallin P*; *n*-propyl gallate; propyl 3,4,5-trihydroxybenzoate; *Tenox PG*

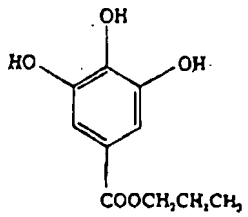
## 3. Chemical Name and CAS Registry Number

3,4,5-Trihydroxybenzoic acid propyl ester
[121-79-9]

## 4. Empirical Formula Molecular Weight

$C_{10}H_{12}O_5$ 212.20

## 5. Structural Formula




## 6. Functional Category

Antioxidant.

## 7. Applications in Pharmaceutical Formulation or Technology

Propyl gallate has become widely used as an antioxidant in cosmetics, perfumes, foods and pharmaceuticals since its use in preventing autoxidation of oils was first described in 1943.[1,2] It is primarily used, in concentrations up to 0.1% w/v, to prevent the rancidity of oils and fats; it may also be used at concentrations of 0.002% w/v to prevent peroxide formation in ether and at 0.01% w/v to prevent the oxidation of paraldehyde. Synergistic effects with other antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene have been reported. Propyl gallate is also said to possess some antimicrobial properties, *see* Section 10.

Other alkyl gallates are also used as antioxidants and have approximately equivalent antioxidant properties when used in equimolar concentration; solubilities however vary, *see* Section 18.

## 8. Description

Propyl gallate is a white, odorless or almost odorless crystalline powder, with a bitter astringent taste which is not normally noticeable at the concentrations employed as an antioxidant.

## 9. Pharmacopeial Specifications

| Test | BP 1993 | USPNF XVII |
|---|---|---|
| Identification | + | + |
| Melting range | 148-151°C | 146-150°C |
| Loss on drying | ≤ 1.0% | ≤ 0.5% |
| Residue on ignition | — | ≤ 0.1% |
| Sulfated ash | ≤ 0.1% | — |
| Chloride | ≤ 330 ppm | — |
| Sulfate | ≤ 0.12% | — |
| Heavy metals | — | ≤ 0.001% |
| Assay (dried basis) | — | 98.0-102.0% |

## 10. Typical Properties

*Antimicrobial activity*: propyl gallate has been reported to possess some antimicrobial activity against Gram-negative, Gram-positive and fungal species.[3] Its effectiveness as a preservative may be improved when used in combination with zinc salts, such as zinc sulfate, due to synergistic effects.[4] Reported minimum inhibitory concentrations (MICs) for aqueous solutions containing 4% v/v ethanol as cosolvent are shown below:[3]

| Microorganism | MIC (μg/mL) |
|---|---|
| *Candida albicans* | 1500 |
| *Escherichia coli* | 330 |
| *Staphylococcus aureus* | 600 |

*Melting point*: 150°C
*Solubility*:

| Solvent | Solubility at 20°C Unless otherwise stated |
|---|---|
| Almond oil | 1 in 44 |
| Castor oil | 1 in 4.5 |
| Cottonseed oil | 1 in 81 at 30°C |
| Ethanol (95%) | 1 in 3 |
| | 1 in 0.98 at 25°C |
| Ether | 1 in 3 |
| | 1 in 1.2 at 25°C |
| Lanolin | 1 in 16.7 at 25°C |
| Lard | 1 in 88 at 45°C |
| Mineral oil | 1 in 200 |
| Peanut oil | 1 in 2000 |
| Propylene glycol | 1 in 2.5 at 25°C |
| Soybean oil | 1 in 100 at 25°C |
| Water | 1 in 1000 |
| | 1 in 286 at 25°C |

## 11. Stability and Storage Conditions

Propyl gallate is unstable at high temperatures and is rapidly destroyed in oils that are used for frying purposes.

The bulk material should be stored in a well-closed, nonmetallic container, protected from light, in a cool, dry, place.

## 12. Incompatibilities

The alkyl gallates, are incompatible with metals, e.g. sodium, potassium and iron, forming intensely colored complexes. Complex formation may be prevented, under some circumstances, by the addition of a sequestering agent, typically citric acid. Propyl gallate may also react with oxidizing materials.

## 13. Method of Manufacture

Propyl gallate is prepared by the esterification of 3,4,5-trihydroxybenzoic acid (gallic acid) with *n*-propanol. Other alkyl gallates are similarly prepared using an appropriate alcohol of the desired alkyl chain length.

## 14. Safety

It has been reported, following animal studies, that propyl gallate has a strong contact sensitization potential.[5] However, despite this, there have been few reports of adverse reactions.[6] Those that have been described include: contact dermatitis; : gic contact dermatitis;[7] and methemoglobinemia in neonates.[8]

The WHO has set an estimated acceptable daily intake for propyl gallate at up to 2.5 mg/kg body-weight.[9]

$LD_{50}$ (cat, oral): 0.4 g/kg[10]
$LD_{50}$ (mouse, oral): 1.7 g/kg
$LD_{50}$ (rat, oral): 3.8 g/kg
$LD_{50}$ (rat, IP): 0.38 g/kg

## 15. Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Eye protection and gloves are recommended. When heated to decomposition propyl gallate may emit toxic fumes and smoke.

## 16. Regulatory Status

GRAS listed. Accepted for use as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (IM injections and topical preparations). Included in nonparenteral medicines licensed in the UK.

## 17. Pharmacopeias

A··, Br, Cz, Egypt, Fr, Ind, Nord and USPNF

## 18. Related Substances

Dodecyl gallate; ethyl gallate; octyl gallate.

**Dodecyl gallate:** $C_{19}H_{30}O_5$
*Molecular weight*: 338.44
*CAS number*: [1166-52-5]
*Synonyms*: dodecyl 3,4,5-trihydroxybenzoate; E312; lauryl gallate.
*Pharmacopeias*: Aust, Br and Fr.
*Appearance*: white, odorless or almost odorless crystalline powder.
*Melting point*: 96-97.5°C
*Solubility*:

| Solvent | Solubility at 20°C |
|---|---|
| Acetone | 1 in 2 |
| Chloroform | 1 in 60 |
| Ethanol (95%) | 1 in 3.5 |
| Ether | 1 in 4 |
| Methanol | 1 in 1.5 |
| Peanut oil | 1 in 30 |
| Propylene glycol | 1 in 60 |
| Water | practically insoluble |

**Ethyl gallate:** $C_9H_{10}O_5$
*Molecular weight*: 198.17
*CAS number*: [831-61-8]
*Synonyms*: ethyl 3,4,5-trihydroxybenzoate.
*Pharmacopeias*: Br.
*Appearance*: white, odorless or almost odorless, crystalline powder.
*Melting point*: 151-154°C
*Solubility*:

| Solvent | Solubility at 20°C |
|---|---|
| Ethanol (95%) | 1 in 3 |
| Ether | 1 in 3 |
| Peanut oil | practically insoluble |
| Water | slightly soluble |

**Octyl gallate:** $C_{15}H_{22}O_5$
*Molecular weight*: 282.34
*CAS number*: [1034-01-1]
*Synonyms*: E311; octyl 3,4,5-trihydroxybenzoate.
*Pharmacopeias*: Br and Fr.
*Appearance*: white, odorless or almost odorless crystalline powder.
*Melting point*: 100-102°C
*Solubility*:

| Solvent | Solubility at 20°C |
|---|---|
| Acetone | 1 in 1 |
| Chloroform | 1 in 30 |
| Ethanol (95%) | 1 in 2.5 |
| Ether | 1 in 3 |
| Methanol | 1 in 0.7 |
| Peanut oil | 1 in 33 |
| Propylene glycol | 1 in 7 |
| Water | practically insoluble |

## 19. Comments

Propyl gallate has been reported to impart an 'off' flavor to corn and cottonseed oils when used as an antioxidant.[11] An acceptable daily intake for dodecyl gallate and octyl gallate was not set by the WHO due to insufficient data. The use of octyl gallate in beer and other widely consumed beverages was however not recommended by the WHO due to the possibility of adverse reactions in the buccal mucosa of individuals previously sensitized by cutaneous contact with this compound.[9]

## 20. Specific References


1. Boehm E, Williams R. The action of propyl gallate on the autoxidation of oils. Pharm J 1943; 151: 53.
2. Boehm E, Williams R. A study of the inhibiting actions of propyl gallate (normal propyl trihydroxy benzoate) and certain other trihydric phenols on the autoxidation of animal and vegetable oils. Chemist Drugg 1943; 140: 146-147.
3. Zeelie JJ, McCarthy TJ. The potential antimicrobial properties of antioxidants in pharmaceutical systems. S Afr Pharm J 1982; 49: 552-554.
4. McCarthy TJ, Zeelie JJ, Krause DJ. The antimicrobial action of zinc ion/antioxidant combinations. J Clin Pharm Ther 1992; 17: 51-54.

5. Kahn G, Phanuphak P, Claman HN. Propyl gallate contact sensitization and orally induced tolerance. Arch Dermatol 1979, 104. 506-509.
6. Golightly LK, Smolinske SS, Bennett ML, Sutherland EW, Rumack BH Pharmaceutical excipients: adverse effects associated with 'inactive' ingredients in drug products (part II) Med Toxicol 1988; 3: 209-240.
7. Bojs G, Nicklasson B, Svensson A. Allergic contact dermatitis to propyl gallate Contact Dermatitis 1987, 17: 294-298.
8. Nitzan M, Volovitz B, Topper E. Infantile methemoglobinemia caused by food additives. Clin Toxicol 1979; 15(3): 273-280
9. FAO/WHO. Evaluation of certain food additives and contaminants: thirteenth report of the joint FAO/WHO expert committee on food additives Tech Rep Ser Wld Hlth Org 1987; No. 751
10. Sweet DV, editor. Registry of toxic effects of chemical substances Cincinnati: US Department of Health, 1987
11. McConnell JEW, Esselen WB Effect of storage conditions and antioxidants on the keeping quality of packaged oils. J Am Oil Chem Soc 1947; 24. 6-14.

## 21. General References

Johnson DM, Gu LC. Autoxidation and antioxidants. In. Swarbrick J, Boylan JC, editors. Encyclopedia of pharmaceutical technology, volume 1. New York: Marcel Dekker Inc, 1988: 415-449.

## 22. Authors

UK: PJ Weller.

# Butylated Hydroxytoluene

## 1. Nonproprietary Names

BP: Butylated hydroxytoluene
PhEur: Butylhydroxytoluenum
USPNF: Butylated hydroxytoluene

## 2. Synonyms

*Advastab-401*; *Agidol*; *Annulex BHT*; *Antioxidant 30*; *Antrancine 8*; BHT; 2,6-bis(1,1-dimethylethyl)-4-methylphenol; butylhydroxytoluene; *Dalpac*; dibutylated hydroxytoluene; 2,6-di-*tert*-butyl-*p*-cresol; 3,5-di-*tert*-butyl-4-hydroxytoluene; E321; *Embanox BHT*; *Impruvol*; *Ionol CP*; *Nipanox BHT*; *OHS28890*; *Sustane*; *Tenox BHT*; *Topanol*; *Vianol*.

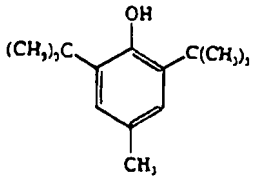
## 3. Chemical Name and CAS Registry Number

2,6-Di-*tert*-butyl-4-methylphenol [128-37-0]

## 4. Empirical Formula and Molecular Weight

$C_{15}H_{24}O$ 220.35

## 5. Structural Formula




## 6. Functional Category

Antioxidant.

## 7. Applications in Pharmaceutical Formulation or Technology

Butylated hydroxytoluene is used as an antioxidant in cosmetics, foods and pharmaceuticals. It is mainly used to delay or prevent oxidative rancidity of fats and oils and to prevent loss of activity of oil-soluble vitamins. Butylated hydroxytoluene is also used at 0.5-1% concentration in natural or synthetic rubber to provide enhanced color stability.
Butylated hydroxytoluene has some antiviral activity[1] and has been used therapeutically to treat herpes simplex labialis.[2]

| Antioxidant use | Concentration (%) |
|---|---|
| β-Carotene | 0.01 |
| Edible vegetable oils | 0.01 |
| Essential oils and flavoring agents | 0.02-0.5 |
| Fats and oils | 0.02 |
| Fish oils | 0.01-0.1 |
| Inhalations | 0.01 |
| IM injections | 0.03 |
| IV injections | 0.0009-0.002 |
| Topical formulations | 0.0075-0.1 |
| Vitamin A | 10 mg per million units |

## 8. Description

Butylated hydroxytoluene occurs as a white or pale yellow crystalline solid or powder with a faint characteristic odor.

## 9. Pharmacopeial Specifications

| Test | PhEur 1988 | USPNF XVII |
|---|---|---|
| Identification | + | + |
| Appearance of solution | + | – |
| Congealing temperature | – | ≥ 69.2°C |
| Freezing-point | 69-70°C | – |
| Residue on ignition | – | ≤ 0.002% |
| Sulfated ash | ≤ 0.1% | – |
| Arsenic | – | ≤ 3 ppm |
| Heavy metals | – | ≤ 0.001% |
| Related substances | + | – |
| Assay | – | ≥ 99.0% |

## 10. Typical Properties

*Boiling point*: 265°C
*Density (bulk)*: 0.48-0.60 g/cm$^3$
*Flash point*: 127°C (open cup)
*Latent heat of fusion*: 23.4 J/g (16.5 cal/g)
*Melting point*: 70°C
*Partition coefficients*: Octanol: water = 4.17-5.80
*Refractive index*: $n_D^{75}$ = 1.4859
*Solubility*: practically insoluble in water, glycerin, propylene glycol, solutions of alkali hydroxides and dilute aqueous mineral acids. Freely soluble in acetone, benzene, ethanol (95%), ether, methanol, toluene, fixed oils and liquid paraffin. More soluble in food oils and fats than butylated hydroxyanisole. *See also* HPE Data.
*Specific gravity*:
1.006 at 20°C;
0.890 at 80°C;
0.883 at 90°C;
0.800 at 100°C.
*Specific heat*:
1.63 J/g/°C (0.39 cal/g/°C) for solid;
2.05 J/g/°C (0.49 cal/g/°C) for liquid.
*Vapor density (relative)*: 7.6 (air = 1)
*Vapor pressure*:
1.33 Pa (0.01 mmHg) at 20°C;
266.6 Pa (2 mmHg) at 100°C.
*Viscosity (kinematic)*: 3.47 mm$^2$/s (3.47 cSt) at 80°C

| | HPE Laboratory Project Data | | |
|---|---|---|---|
| | Method | Lab # | Results |
| Density | DE-1 | 31 | 1.031 g/cm$^3$ |
| Solubility | | | |
| Ethanol (95%) at 25°C | SOL-7 | 32 | 108 mg/mL |
| Ethanol (95%) at 37°C | SOL-7 | 32 | 147 mg/mL |
| Hexane at 25°C | SOL-7 | 32 | 409 mg/mL |
| Hexane at 37°C | SOL-7 | 32 | 514 mg/mL |
| Propylene glycol at 25°C | SOL-7 | 32 | Insoluble |
| Propylene glycol at 37°C | SOL-7 | 32 | Insoluble |
| Water at 25°C | SOL-7 | 32 | Insoluble |
| Water at 37°C | SOL-7 | 32 | Insoluble |

Supplier: Koppers Company Inc.

## 11. Stability and Storage Conditions

Exposure to light, moisture and heat cause discoloration and a loss of activity. Butylated hydroxytoluene should be stored in a

well-closed container, protected from light, in a cool, dry, place.

## 12. Incompatibilities

Butylated hydroxytoluene is phenolic and undergoes reactions characteristic of phenols. It is incompatible with strong oxidizing agents such as peroxides and permanganates. Iron salts cause discoloration with loss of activity. Heating with catalytic amounts of acids causes rapid decomposition with the release of the flammable gas isobutylene.

## 13. Method of Manufacture

Prepared by the reaction of *p*-cresol with isobutylene.

## 14. Safety

Butylated hydroxytoluene is readily absorbed from the gastrointestinal tract and is metabolized and excreted in the urine mainly as glucuronide conjugates of oxidation products. Although there have been some isolated reports of adverse skin reactions, butylated hydroxytoluene is generally regarded as nonirritant and nonsensitizing at the levels employed as an antioxidant.[3,4]

The WHO has set a temporary estimated acceptable daily intake for butylated hydroxytoluene at up to 125 μg/kg body-weight.[5]

Ingestion of 4 g of butylated hydroxytoluene, although causing severe nausea and vomiting, has been reported to be nonfatal.[6]

$LD_{50}$ (guinea pig, oral): 6.4-12.8 g/kg[7]
$LD_{50}$ (mouse, IP): 0.14 g/kg
$LD_{50}$ (mouse, IV): 0.18 g/kg
$LD_{50}$ (mouse, oral): 0.8-1.6 g/kg
$LD_{50}$ (rat, oral): 0.89 g/kg

## 15. Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Butylated hydroxyanisole may be irritant to the eyes, skin, and on inhalation. It should be handled in a well-ventilated environment; gloves and eye protection are recommended.

## 16. Regulatory Status

GRAS listed. Accepted as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (inhalations, IM and IV injections, oral capsules and tablets, rectal, topical and vaginal preparations). Included in nonparenteral medicines licensed in the UK.

## 17. Pharmacopeias

Br, Eur, Fr, Ger, Ind, Mex, Neth, Nord, Swiss and USPNF.

## 18. Related Substances

Butylated Hydroxyanisole.

## 19. Comments

—

## 20. Specific References


1. Snipes W, et al. Butylated hydroxytoluene inactivates lipid-containing viruses. Science 1975; 188: 64-66
2. Freeman DJ, Wenerstrom G, Spruance SL. Treatment of recurrent herpes simplex labialis with topical butylated hydroxytoluene. Clin Pharmacol Ther 1985; 38: 56-59.
3. Roed-Peterson J, Hjorth N. Contact dermatitis from antioxidants: hidden sensitizers in topical medications and foods. Br J Dermatol 1976, 94: 233-241.
4. Juhlin L. Recurrent urticaria: clinical investigation of 330 patients. Br J Dermatol 1981, 104: 369-381.
5. FAO/WHO. Evaluation of certain food additives and contaminants. Thirty-seventh report of the joint FAO/WHO expert committee on food additives. Tech Rep Ser Wld Hlth Org 1991, No. 806.
6. Shlian DM, Goldstone J. Toxicity of butylated hydroxytoluene. N Eng J Med 1986; 314: 648-649
7. Sweet DV, editor. Registry of toxic effects of chemical substances. Cincinnati. US Department of Health, 1987


## 21. General References


Verhagen H. Toxicology of the food additives BHA and BHT. Pharm Weekbl (Sci) 1990; 12: 164-166.


## 22. Authors


USA: MJ Groves.

# Butylated Hydroxyanisole

## 1. Nonproprietary Names

BP: Butylated hydroxyanisole
USPNF: Butylated hydroxyanisole

## 2. Synonyms

*Antrancine 12*; BHA, *tert*-butyl-4-methoxyphenol; 1,1-dimethylethyl-4-methoxyphenol; E320; *Embanox BHA*; *Nipanox BHA*; *Nipantiox 1-F*; *PM 1787*; *PM 1788*; *PM 12366*; *Sustane 1-F*; *Tenox BHA*.

## 3. Chemical Name and CAS Registry Number

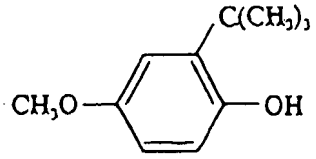
2-*tert*-Butyl-4-methoxyphenol [25013-16-5]

## 4. Empirical Formula Molecular Weight

$C_{11}H_{16}O_2$ 180.25

The BP 1993 describes butylated hydroxyanisole as 2-*tert*-butyl-4-methoxyphenol containing a variable amount of 3-*tert*-butyl-4-methoxyphenol.

## 5. Structural Formula




## Functional Category

Antioxidant.

## 7. Applications in Pharmaceutical Formulation or Technology

Butylated hydroxyanisole is an antioxidant with some antimicrobial properties.[1] It is used in cosmetics, foods and pharmaceuticals particularly to delay or prevent oxidative rancidity of fats and oils and to prevent loss of activity of oil-soluble vitamins.

Butylated hydroxyanisole is frequently used in combination with other antioxidants, particularly butylated hydroxytoluene and alkyl gallates, and with sequestrants or synergists such as citric acid.

| Antioxidant use | Concentration (%) |
|---|---|
| β-Carotene | 0.01 |
| Essential oils and flavoring agents | 0.02-0.5 |
| IM injections | 0.03 |
| IV injections | 0.0002-0.0005 |
| Oils and fats | 0.02 |
| Topical formulations | 0.005-0.02 |
| Vitamin A | 10 mg per million units |

## 8. Description

Butylated hydroxyanisole occurs as a white or almost white crystalline powder or a yellowish-white waxy solid with a faint, characteristic aromatic odor.

## 9. Pharmacopeial Specifications

| Test | BP 1993 (Ad 1994) | USPNF XVII (Suppl 6) |
|---|---|---|
| Identification | + | + |
| Residue on ignition | — | ≤ 0.01% |
| Sulfated ash | ≤ 0.05% | — |
| Related substances | + | — |
| Arsenic | — | ≤ 3 ppm |
| Heavy metals | — | ≤ 0.001% |
| Organic volatile matter | — | + |
| Assay | — | ≥ 98.5% |

## 10. Typical Properties

*Antimicrobial activity*: activity is similar to that of the *p*-hydroxybenzoate esters (parabens). The greatest activity is against molds and Gram-positive bacteria, with less activity against Gram-negative bacteria.

*Boiling point*: 264°C

*Melting point*: 47°C (for pure 2-*tert*-butyl-4-methoxyphenol), *see also* Section 19.

*Solubility*: practically insoluble in water; freely soluble in ≥ 50% aqueous ethanol, propylene glycol, chloroform, ether, hexane, cottonseed oil, peanut oil, soybean oil and in solutions of alkali hydroxides. *See also* HPE Data.

*Specific gravity*: 1.05 at 20°C

*Viscosity (kinematic)*: 3.3 $mm^2/s$ (3.3 cSt) at 99°C

| | HPE Laboratory Project Data | | |
|---|---|---|---|
| | Method | Lab # | Results |
| Density | DE-1 | 31 | 1.117 $g/cm^3$ |
| Solubility | | | |
| Ethanol (95%) at 25°C | SOL-7 | 32 | 793.0 mg/mL |
| Ethanol (95%) at 37°C | SOL-7 | 32 | 834.0 mg/mL |
| Hexane at 25°C | SOL-7 | 32 | 48.0 mg/mL |
| Hexane at 37°C | SOL-7 | 32 | 10.0 mg/mL |
| Propylene glycol at 25°C | SOL-7 | 32 | 467.0 mg/mL |
| Propylene glycol at 37°C | SOL-7 | 32 | 456.0 mg/mL |
| Water at 25°C | SOL-7 | 32 | 0.32 mg/mL |
| Water at 37°C | SOL-7 | 32 | 0.78 mg/mL |

Supplier: Eastman Fine Chemicals.

## 11. Stability and Storage Conditions

Exposure to light causes discoloration and loss of activity. Butylated hydroxyanisole should be stored in a well-closed container, protected from light, in a cool, dry, place.

## 12. Incompatibilities

Butylated hydroxyanisole is phenolic and undergoes reactions characteristic of phenols. It is incompatible with oxidizing agents and ferric salts. Trace quantities of metals, and exposure to light, cause discoloration and loss of activity.

## 13. Method of Manufacture

Prepared by the reaction of *p*-methoxyphenol with isobutene.

## 14. Safety

Butylated hydroxyanisole is absorbed from the gastrointestinal tract and is metabolized and excreted in the urine with less than 1% unchanged within 24 hours of ingestion.[2] Although there have been some isolated reports of adverse skin reactions to butylated hydroxyanisole[3,4] it is generally regarded as nonirritant and nonsensitizing at the levels employed as an antioxidant.

Concern over the use of butylated hydroxyanisole has occurred following long-term animal feeding studies. Although previous studies in rats and mice fed butylated hydroxyanisole at several hundred times the US permitted level in the human diet showed no adverse effects, a study in which rats, hamsters and mice were fed butylated hydroxyanisole at 1-2% of the diet produced benign and malignant tumors of the forestomach, but in no other sites. However, humans do not have any region of the stomach comparable to the rodent forestomach and studies in animals that also do not have a comparable organ (dogs, monkeys and guinea pigs) showed no adverse effects. Thus, the weight of evidence does not support any relevance to the human diet where butylated hydroxyanisole is ingested at much lower levels.[5] The WHO acceptable daily intake of butylated hydroxyanisole has been set at 500 μg/kg body-weight.[5]

$LD_{50}$ (mouse, oral): 2.0 g/kg[6]
$LD_{50}$ (rat, IP): 0.88 g/kg
$LD_{50}$ (rat, oral): 2.2 g/kg

## 15. Handling Precautions

Observe normal precautions appropriate to the circumstances and quantity of material handled. Butylated hydroxyanisole may be irritant to the eyes, skin, and on inhalation. It should be handled in a well-ventilated environment; gloves and eye protection are recommended.

## 16. Regulatory Status

GRAS listed. Accepted as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (inhalations, IM and IV injections, oral capsules and tablets, rectal, topical and vaginal preparations). Included in nonparenteral medicines licensed in the UK.

## 17. Pharmacopeias

Br, Fr, Ind, It, Mex and USPNF.

## 18. Related Substances

Butylated Hydroxytoluene

## 19. Comments

The commercially available material can have a wide melting point range (47-57°C) due to the presence of varying amounts of 3-*tert*-butyl-4-methoxyphenol.

*Tenox* brands contain 0.1% w/w citric acid as a stabilizer.

## 20. Specific References


1. Lamikanra A, Ogunbayo TA. A study of the antibacterial activity of butyl hydroxy anisole (BHA). Cosmet Toilet 1985; 100(10): 69-74.
2. El-Rashidy R, Niazi S. A new metabolite of butylated hydroxyanisole in man. Biopharm Drug Dispos 1983; 4: 389-396.
3. Roed-Peterson J, Hjorth N. Contact dermatitis from antioxidants: hidden sensitizers in topical medications and foods. Br J Dermatol 1976; 94: 233-241.
4. Juhlin L. Recurrent urticaria: clinical investigation of 330 patients. Br J Dermatol 1981; 104: 369-381.
5. FAO/WHO. Evaluation of certain food additives and contaminants. Thirty-third report of the joint FAO/WHO expert committee on food additives. Tech Rep Ser Wld Hlth Org 1989, No. 776.
6. Sweet DV, editor. Registry of toxic effects of chemical substances. Cincinnati: US Department of Health; 1987.


## 21. General References


Babich H, Borenfreund E. Cytotoxic effects of food additives and pharmaceuticals on cells in culture as determined with the neutral red assay. J Pharm Sci 1990; 79: 592-594.

Verhagen H. Toxicology of the food additives BHA and BHT. Pharm Weekbl Sci 1990; 12: 164-166


## 22. Authors


USA: MJ Groves.